Zlinská, J., Šomšák, L., Holecová, M., 2005: Ecological characteristics of studied forest communities of an oak-hornbeam tier in SW Slovakia. Ekológia (Bratislava), *24*, Suppl. 2, p. 3–19.

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Mrva M.: **Diverzita nahých meňaviek** (Rhizopoda, Gymnamoebia) **v machoch Malých Karpát (Slovensko).**

Počas rokov 2000–2002 som študoval faunu nahých meňaviek (Rhizopoda, Gymnamoebia) machov na piatich lokalitách dubovo-hrabových lesov Malých Karpát. Vysušený materiál som navlhčil destilovanou vodou a po piatich dňoch kultivácie som sledoval zastúpenie meňaviek. Determinácia prebehla na základe morfologických kritérií aktívnych štádií. Zaznamenal som pomerne vysokú celkovú diverzitu 32 taxónov nahých meňaviek, ktorá sa pohybovala na jednotlivých lokalitách od 17 po 23 taxónov. Najviac bola druhovo zastúpená čeľaď Thecamoebidae (9 druhov), pomerne vysoké počty druhov boli zistené aj u čeľadí Hartmannellidae, Vannellidae a Paramoebidae. Zistené druhové zastúpenie nasvedčuje tomu, že spoločenstvo nahých meňaviek v skúmaných machoch je podobné spoločenstvám v sladkovodných biotopoch.

COMMUNITY STRUCTURE AND ECOLOGICAL MACRODISTRIBUTION OF MOSS-DWELLING WATER BEARS (Tardigrada) IN CENTRAL EUROPEAN OAK-HORNBEAM FORESTS (SW SLOVAKIA)

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Abstract

Degma P., Šimurka M., Gulánová S.: Community structure and ecological macrodistribution of moss-dwelling water bears (Tardigrada) in Central European oak-hornbeam forests (SW Slovakia). Ekológia (Bratislava), Vol. 24, Supplement 2/2005, p. 59–75.

The structure of tardigrade communities in mosses of Central European oak-hornbeam forests was studied at 10 sites located in the Malé Karpaty Mts and Trnavská pahorkatina hills (SW Slovakia). A total of 3, 050 tardigrade specimens of 21 species and 2 families were gathered from 79 quantitative samples taken from 2000 and 2002.

Kruskal-Wallis' tests and regression analyses showed no statistically significant influence between the 12 studied environmental variables and the number of Tardigrada specimens or number of species in the samples. A chi-square goodness of fit test suggested that the number of Tardigrada species in samples of moss *Hypnum cupressiforme* was random within the investigated area.

A t-test of tardigrade species diversity resulted in significant differences between study sites. The group of communities with the lowest diversity does not differ mutually. Majority of differences in species diversity were caused by randomly found species.

Results of cluster analysis as well as CCA point out that distribution of tardigrades and their colonisation of particular substrata is a random process.

Although the results are affected by restricted number of samples, we believe that Tardigrada as passively dispersed organisms are without significant relationship to ecological variables related to their distribution amongst substrata.

Key words: Tardigrada, community structure, ecological macrodistribution, mosses, oak-hornbeam forests, Central Europe

Introduction

Tardigrades living in mosses are organisms passively dispersed predominantly by wind but also by rain, floodwaters and melting snow. Occasionally animals visiting mosses can transport their specimens or eggs. Density of populations in colonized substrata can depend on several abiotic and biotic factors (Ramazzotti, Maucci, 1983).

Community structure or ecological macrodistribution (distribution between substrata) of moss-dwelling tardigrades have been already discussed in more papers (Dastych, 1988; Ito, 1999; Peters, Dumjahn, 1999 etc.). Although these studies were based on quantitative data only few of them have used statistical methods so far (Nelson, 1975; Kathman, Cross, 1991; Wright, 1991; Nelson, Adkins, 2001; Nichols et al., 2001; Romano et al., 2001; Jönsson, 2003).

The present study is a part of the project focused on microfauna and arthropod communities in oak-hornbeam forests (predominantly in soil) in SW Slovakia. From moss fauna, only tardigrades and active gymnamoebae (Mrva, 2005) were studied within this project. Only one study (Guoth, 1986) and several published records including the description of *Echiniscus pajstunensis* Bartoš, 1941 have referred to Tardigrada of this region (Bartoš, 1937a,b, 1941; Degma, 2003; Degma et al., 2004, 2005).

The goal of this study is to characterize the structure of the tardigrade community in mosses of Central European oak-hornbeam forests using the data from SW Slovakia as well as to identify significant environmental variables which affect the structure.

Material and methods

All the studied taxonomic groups including Tardigrada were sampled at the following study plots located in the Malé Karpaty Mts and Trnavská pahorkatina hills (SW Slovakia): Cajla, Vinosady, Fúgelka, Lindava Nature Reserve, Horný háj grove, Lošonec–lom quarry, Lošonský háj grove Nature Reserve, Naháč–Kukovačník, Naháč–Katarínka 1 Nature Reserve, Naháč–Katarínka 2 Nature Reserve (to locate study plots and their characterization see Zlinská et al., 2005).

Quantitative samples of mosses (79 samples) were taken from each study plot in two periods: in 2000 (May, 31 or August, 15) and in 2002 (November, 7 or November, 8). Each quantitative sample was cut from substrate using metallic cylinder with surface of 10 cm^2 with minimal addition of soil. Eight samples were taken from each the study plot with one exception (for number of samples in each study plot see Table 3). Immediately after cutting, each sample was put into a labelled paper bag for gradual drying.

Laboratory processing of the samples also followed the procedure according to Dastych (1980) including submersion of a sample in a tap water for a period of 20–24 hours, detaching material from a substrate by agitation, squeezing and sedimentation. Extraction of tardigrades, their eggs and exuvia from sediment in Petri dish was done under the stereo-microscope (magnification 40x) using a micropipette. Specimens were mounted in Hoyer's medium and the presence or an absence of eyes was noted before they dissolved in medium. Cover slips were sealed with asphalt varnish after medium desiccation.

Specimens were identified under light microscope with phase contrast and oil immersion using species descriptions of Ramazzotti, Maucci (1983) as translated by Beasley (1995), original species descriptions and using the keys by Biserov (1990), Bertolani, Rebecchi (1993), Pilato, Binda (1999) and Pilato et al. (2000). Specimens of the genus *Macrobiotus* remained unidentified when their eggs were absent or only simplex specimens were found in a sample. Some specimens of the genera *Hypsibius* and *Diphascon* also remained unidentified as only simplex specimens were found.

Environmental variables were measured or noted at each study plot (Zlinská et al. 2005) and the values 9 of them were used for comparisons (Table 1). Three additional variables were noted for each sample (Table 2).

Table 1. Values of chosen environmental variables at different study plots.

Study plot		Altitude [m a.s.l]	Exposition	Slope [°]	Forest stand age [years]	E_1 cover [%]	E_2 cover [%]	E_3 cover [%]	pH of litter in H_2O	Forest fragmentation [%]
CA	Cajla	270	S	7	80–100	90	2	75	4.83	20
VI	Vinosady	280	NW	6	60–80	80	30	70	4.48	30
FU	Fúgelka	350	S	5	80–100	40	50	80	3.90	10
LI	Lindava Nature Reserve	240	none	0	80–100	80	1	65	4.68	0
HH	Horný háj grove	240	W-SW	5	60–80	75	10	80	5.00	15
LL	Lošonec–lom quarry	340	SW	9	80–100	100	25	65	6.74	5
LH	Lošonský háj grove Nature Reserve	260	NE	1	80–100	75	1	80	4.29	15
NA	Naháč–Kukovačník	300	NE	3	40–60	100	20	75	4.18	50
NK1	Naháč–Katarínka 1 Nature Reserve	340	NW	4	40–60	80	1	80	4.24	0
NK2	Naháč–Katarínka 2 Nature Reserve	320	SE	45	100–120	90	35	75	6.45	20

Constancy is defined as the number of study plots where the tardigrade species was found divided by total number of study plots and expressed as a percentage. Frequency is defined as the number of samples in which the tardigrade species occurred in the entire studied area divided by the total number of samples and also expressed as a percentage (Nelson, 1975). The calculation of Tardigrada species dominancy in study plots as well as in the whole area was based on all specimens found including the unidentified ones. Species diversity was measured using Shannon-Wiener's index (natural logarithms were used) and the diversity of each community at the study sites was compared with a t-test (Poole, 1974; Spellerberg, Fedor, 2003).

Kruskal-Wallis' tests were done to evaluate the impact of nominal variables on the total number of Tardigrada specimens as well as on the number of species at the sites, underbeds, study plot exposure, age of a forest stand and month of sampling using all 79 samples. Species of mosses did not enter into any Kruskal-Wallis' test as their distribution between the samples was very unequal, most samples –54 of them– were taken from the dominant species *Hypnum cupressiforme* and number of samples taken from rest of moss species varied from 1 to 5. Chi-square Goodness of fit test with Poisson distribution was performed to test whether Tardigrada species in the samples of moss *Hypnum cupressiforme* were distributed randomly in the studied area. Simple linear regression analyses were done to test relationships between dependent number of tardigrade specimens or number of their species in all 79 samples and each of 7 gradient variables. Stepwise regression analysis with forward selection of gradient variables was performed to identify variables with significant influence on the number of specimens or the number of species in the samples. Kruskal-Wallis's tests as well as regression analyses and Chi-square Goodness of fit test were performed using computer programme Statgraphics Plus V.7.0 (Manugistics, 1993).

The cluster analysis of the communities at all the study sites were done using the computer program Nclas from the package Syn-Tax (Podani, 1993). The complete linkage clustering method in combination with Wishart's similarity ratio index for clustering according to abundance of species transformed with log-transformation was used.

Canonical Correspondence Analysis (CCA) with forward selection of environmental variables using Monte Carlo permutation tests for significance of each variable as well as for significance of the first canonical axis was performed using the program Canoco (ter Braak, Šmilauer, 1998). The goal was to find

Table 2. Number of samples per each additional variable category.

Variable	Category of variable / Study plot	CA	VI	FU	LI	HH	LL	LH	NA	NK1	NK2
Underbed	soil	1	1	1	4	3	0	5	1	4	0
	rock	2	0	2	0	2	1*	0	0	0	6
	living tree	4	5	3	2	1	2*	0	4	0	1
	dead tree	1	2	2	2	2	4*	3	3	4	0
Moss species	*Amblystegium serpens* (H e d w.) B. S. G.			**		**					**
	Anomodon attenuatus (H e d w.) H u e b e n e r					1					1
	A. viticulosus (H e d w.) H o o k et T a y l o r					1					1
	Atrichum undulatum (H e d w.) P. B e a u v.		1								
	Brachythecium populeum (H e d w.) B. S. G.						1				
	B. rutabulum (Hedw.) B. S. G.			**		1	1	7		1	1
	B. velutinum (Hedw.) B. S. G.					**					
	Cirriphyllum tommasinii (S e n d t n. ex B o u l a y) G r o u t										1
	Dicranum scoparium H e d w.				**					1	
	Homalothecium lutescens (H e d w.) H. R o b.					1					
	H. sericeum (Hedw.) B. S. G.					1					
	Hypnum cupressiforme H e d w.	7	7	6	6	2	6		7	5	1
	Plagiomnium rostratum (S c h r a d.) T. J. K o p.										1
	Plagiothecium sp.				**				1		
	Polytrichum formosum H e d w.	1		1	1 and **			1		1	
	Pseudoleskeella nervosa (B r i d.) N y h o l m										**
Sampling month	May	5	4	4	6	3	0	0	0	0	0
	August	0	0	0	0	0	5	3	3	3	4
	November	3	4	4	2	5	3	5	5	5	3

Notes: * underbed was not noted for one sample; ** composite sample from more moss species; for abbreviations of study plots see Table 1

significant environmental variables influencing the structure of the Tardigrada community. Pooled abundance of each species at particular study plots transformed with log-transformation Y = log (Y + 1) entered this analysis together with the values of 9 environmental variables from the 13 mentioned above. Variables 'study plot' (defined analysed samples) as well as underbed, substrate species and month of sampling were omitted from the analysis (the later three ones had various values in particular samples). CCA was also performed for examination of the relationship between Tardigrada species and all 13 environmental variables to identify significant ones influencing species assemblages in the 79 samples expressed by transformed abundance of each species with log-transformation. Downweighting of rare species was used to suppress the influence of randomly found species that were collected in low numbers on the overall result of this analysis.

Only the identified specimens were taken into all the statistical analyses with exception of Kruskal-Wallis' tests and regression analyses when total numbers of all Tardigrada specimens in the samples were involved. Nomenclature of tardigrades is according to Guidetti, Bertolani (2005) and that of mosses according to Kubinska, Mišíková (1998).

Results and discussion

Structure of Tardigrada *communities in oak-hornbeam forests*

Abundancy and number of Tardigrada species

A total of 3, 050 specimens of water bears were found and identified except for 164 (5.4%) which lacked eggs or were only found in simplex stage. One class, 2 families and 21 species were represented in the 79 quantitave samples. Tardigrades were absent in 24 (30.4%) of the 79 analysed samples. The number of samples without tardigrades varied between 0 (CA) and 6 (LL, 75% of samples) at the study sites. The lowest number of specimens was recorded in LL (21 specimens in two positive samples) and the highest in CA (953 specimens in 8 positive samples). Minimal numbers of species were detected at study site NA (1 species) and LL (3 species) while maximum species richness was in HH (9 species) (Table 3). The study plot NA is the most isolated (a small forest surrounded by fields) and could be influenced by dust rising during agricultural work. Moreover the site LL was covered with calcareous dust from a near by limestone quarry.

Out of 21 identified species 12 (*Hypsibius convergens, H. dujardini, H. pallidus, Isohypsibius hunulatus, I. prosostomus, Diphascon pingue, D. belgicae, D. prorsirostre, D. scoticum, Astatumen trinacriae, Macrobiotus hufelandi* and *Minibiotus intermedius*) were found at the same altitudes in adjacent Poland. However the Poland species were found in a significantly broader range of altitudes. On the other hand, *Astatumen bartosi* and *Macrobiotus pallarii* were recorded in different altitudinal zones in Poland (Dastych, 1988). We have found only *Isohypsibius hunulatus* from all the upland species which have the center of their distribution in the zone of 201–500 m a.s.l. according to his classification of tardigrades. In the same altitudinal zone in Newfoundland, only seven species were found (Collins, Bateman, 2001). Out of those, four species (*Macrobiotus* cf. *harmsworthi, M. hufelandi, Minibiotus intermedius* and *Diphascon scoticum*) were also recorded at our study sites. Out of the 8 species which prefer (although some of them only slightly) altitudinal zone of 0–500 m a.s.l. based on their frequency in the samples according to Maucci (1981), only *Macrobiotus hufelandi, Minibiotus intermedius,*

Table 3. Dominancy [%] of species at the study sites, their total dominancy (D), constancy (C) and frequency (F).

	Taxon	Study site										D	C	F
		CA	VI	FU	LI	HH	LL	LH	NA	NK1	NK2	[%]	[%]	[%]
	Class: EUTARDIGRADA Richters, 1926													
	Family: Hypsibiidae Pilato, 1969													
1	*Hypsibius convergens* (Urbanowicz, 1925)		12.51		0.39	4.94				0.45		0.8	40.0	7.6
2	*Hypsibius dujardini* (Doyere, 1840)		3.13									0.1	10.0	1.3
3	*Hypsibius* cf. *morikawai* Ito, 1995										5.26	0.1	10.0	1.3
4	*Hypsibius pallidus* Thulin, 1911		78.91					0.28				3.3	20.0	2.5
5	*Isohypsibius lunulatus* (Iharos, 1966)					11.11					7.89	0.4	20.0	5.1
6	*Isohypsibius prosostomus* Thulin, 1928	0.52					4.76			0.45		0.2	30.0	3.8
7	*Diphascon (D.) brevipes* (Marcus, 1936)					1.23					34.21	0.5	20.0	3.8
8	*Diphascon (D.) pingue* (Marcus, 1936)	5.14	3.91	5.79	5.30	4.94		7.93		40.18	18.42	7.9	80.0	26.6
9	*Diphascon (A.) belgicae* Richters, 1911									0.45		0.0	10.0	1.3
10	*Diphascon (A.) prorsirostre* Thulin, 1928	1.05		7.40						2.68		1.3	30.0	6.3
11	*Diphascon (A.) scoticum* Murray, 1905			0.64								0.1	10.0	1.3
12	*Astatumen bartosi* (Weglarska, 1959)										2.63	0.0	10.0	1.3
13	*Astatumen trinacriae* (Arcidiacono, 1962)	0.10		1.29	0.13	3.70	4.76	1.13		0.89		0.5	70.0	10.1
	Family: Macrobiotidae Thulin, 1928													
14	*Macrobiotus* cf. *harmsworthi* Murray, 1907	5.88							96.41	52.68	2.63	11.0	40.0	15.2
15	*Macrobiotus hufelandi* C.A.S. Schultze, 1834	84.99		81.03	82.56	12.34		25.78				59.1	50.0	11.4
16	*Macrobiotus pallarii* Maucci, 1954					11.11		7.93				1.2	20.0	2.5
17	*Macrobiotus* cf. *seychellensis* Biserov, 1994										28.95	0.4	10.0	1.3
18	*Macrobiotus* cf. *vanescens* Pilato et al., 1991					22.22	4.76					0.6	20.0	2.5
19	*Macrobiotus* sp.1			1.61								0.2	10.0	1.3
20	*Minibiotus intermedius* (Plate, 1889)				8.91			39.38		0.45		6.9	30.0	7.6
21	*Minibiotus* sp. 1					1.23						0.0	10.0	1.3
	Total Number of specimens	953	128	311	774	81	21	353	167	224	38			
	Total Number of samples (Nr. of negative ones)	8 (0)	8 (3)	8 (3)	8 (3)	8 (3)	8 (6)	8 (1)	8 (2)	8 (1)	7 (2)			
	Number of moss species	2	2	3	3	6	3	2	2	4	7			

Note: for abbreviations of study plots see Table 1

Hypsibius pallidus and *Diphascon scoticum* were present in our material. Finally, Guoth (1986) identified four species (*Macrobiotus hufelandi*, *Hypsibius dujardini*, *H. microps* and *Diphascon scoticum*) in oak-hornbeam forest in southern parts of the Malé Karpaty Mts (study plot Borinka). Most of Gouth's (1986) species were found in our material as well. It is necessary to say that species like *Hypsibius dujardini* were identified only in a single sample and the limited number of recorded species may be caused by limited samples.

No single tested nominal variable was found significant ($P > 0.05$ for all values of Kruskal-Wallis' statistics) neither for Tardigrada specimens number nor for number of their species when each variable was tested separately by Kruskal-Wallis' test. This result partly reflects relatively small number of values of specimens or species numbers in each category (7–8 values in categories of a study plot, 13–23 values in each underbed category, 7–16 values in study plot exposure categories, 7–40 values in each age of a forest stand and 19–38 values in each month of sampling) as a small number of values makes confidence intervals for median (as well as for mean) broader and these intervals overlap in spite of appropriate mean values are very different from each other. For example average number of specimens in one sample in LL is less than one what is in contradiction with mean in CA when it is more than 116.

Steiner (1994) noticed the highest abundance of Tardigrada in May and a population decline in November and March. The average of Tardigrada quantity in our samples was 52.9 in May, 22.3 in August and 33.3 in November, so as it might seem our research partially confirmed Steiner's findings. It is necessary to mention that the same comment about the differences among study plots can be applied in this case. Due to broad confidence intervals for these medians there is no statistical difference between those seasonal ones in our numbers of specimens per sample. In contradiction to us, Nichols et al. (2001) ascertained higher number of specimens as well as species in spring than in summer and fall of the same year using statistical methods in Alabama. However similarly they did not find a significant difference in these parameters among the sampling stations. The statistical results of Romano et al. (2001) indicated fully inverted distribution as they discovered no significant difference in numbers of Tardigrada specimens during season but these numbers were significantly different among the sampling sites in the other locality in Alabama. These two antagonistic results illustrate how some most different samples – the only sample from spring 1977 in Nichols et al. (2001) and two samples from two sites in Romano et al. (2001) can dramatically influence overall result of statistical analysis. That is why the authors of both the studies emphasized a need of more replicate samples to reduce the significant variability due to random patchiness in tardigrade populations. From these reasons, we do not believe the results of both authors reflect real habitat or seasonal differences.

From all seven gradient variables, only cover of E_2 is significant but only for number of Tardigrada species and not for number of their specimens in moss samples ($F = 4.336$, $P = 0.041$, $R^2 = 5.3\%$) when each variable impact was separately tested by simple linear regression analysis. Coefficient of determination R^2 is too small hence we do not consider even this variable to be significant.

We did not obtain sufficiently good general linear model explaining tardigrade specimen number in the samples when stepwise regression analysis was performed

because the coefficient of determination was too small. For number of species (NS), the best obtained model is NS = 0.0192*E3 – 0.0175*E2 (t = 2.812, P = 0.032 for coefficient of E2; t = 7.585, P < 0.001 for coefficient of E3; F = 40.110, P < 0.001, R^2 = 50.7% for model). As we can see, this model explains only 51% of species number variability in moss samples and so major part of this variability remains unexplained. We think that Tardigrada species number in mosses is either largely random or it is influenced by variables not included into our study. The fact, that altitude does not appear to be important variable for specimen or species number per sample, is not surprising with respect to too small interval of altitudes of our study plots (240–350 m a.s.l.). But interesting is, that the results of two multivariate statistical methods (principal components analysis and cluster analysis) also indicated that the abundance of moss-dwelling tardigrades on Vancouver Island was not dependent upon the altitude (Kathman, Cross, 1991) when they were sampled even in wider range of altitudes (150–1525 m a.s.l.).

In our opinion, the mentioned results indicate that moss-dwelling tardigrades either do not significantly prefer any of the tested nominal variable category or interval of gradient variable values or preference of some categories by any species is statistically balanced by preference of other categories by other species in a community. We could consider these results to support the hypothesis that distribution of Tardigrada on mosses (as well as on other substrata) is a random process and due to their broad ecological valence they survive in colonized substrata whereas their population density and microdistribution can be simply various in time or under influence of some ecological variables. For example, according to Wright (1991) three species – *Macrobiotus hufelandi*, *M. richtersi* and *Isohypsibius prosostomus* are in negative associations. Our samples support this statement as two of three mentioned species (without *Macrobiotus richtersi*) were present in them and they never occurred together. As it concerns tardigrades migration in moss cushion (thus their microdistribution), Wright (1991) discovered vertical migration during dehydration of cushion in *M.richtersi* while according to Nelson, Adkins (2001) migration within the moss cushion was not detected as a result of changes in moisture conditions. Finally, population density may be the duration which elapsed since concrete substratum was colonized by tardigrades.

Number of tardigrade species in samples of moss *Hypnum cupressiforme* from all the study plots varied between 0 and 4. The result of Chi-square Goodness of fit test with Poisson distribution for number of tardigrade species in these samples (χ^2 = 1.198, P = 0.549) allowed us to not reject the null hypothesis that the number of species in moss sample is random and with good agreement with Poisson distribution. So Tardigrada species colonize this moss species cushions in oak-hornbeam forests in sampled area randomly. It is very probable that the same is valid for other substrata with regard to mode of tardigrade distribution. From this reason it is not surprising that the greatest number of tardigrades species was found in the moss species which most samples were taken from. From others moss species, it is notable that even four species of Tardigrada were found in a single sample of *Dicranum scoparium* as well as even seven of them in only two samples of *Anomodon viticulosus*. But this is not in contradiction with our presumption that number of Tardigrada species is random also in these moss species.

This hypothesis can be confirmed or refused only by testing of sufficiently large number of samples.

Dominancy, constancy, frequency and species diversity

Nearly 70% of samples were positive in Tardigrada in the study area while in Poland there were only 29% positive samples in these types of forests (Dastych, 1988).

The most dominant species in the study area were *Macrobiotus hufelandi*, *M.* cf. *harmsworthi*, *Diphascon pingue*, *Minibiotus intermedius* and *Hypsibius pallidus*. But the dominancy of these species varied at different study sites considerably (Table 3). All these species were relatively frequently present in the samples but only four species of all occurred in more than 10% of the samples: *Macrobiotus* cf. *harmsworthi*, *M. hufelandi*, *Diphascon pingue* and *Astatumen trinacriae*. Of them, *Diphascon pingue*, *Astatumen trinacriae* and *Macrobiotus hufelandi* have even the highest constancy values (Table 3).

Species diversity was lowest (beyond that on the study plot NA, when it was equal to zero with respect to only one species found) at the study plot CA and the highest on HH. T-tests of species diversity (Table 4) allowed us to differentiate a group of communities with their diversity as not significantly different. Communities with the lowest species diversity (CA, LI, FU and VI) with 4–6 Tardigrada species present and simultaneously one of them having very high dominancy (around 80%) belonged into this group. *M. hufelandi* was the dominating species at all the mentioned study plots with exception for the study plot VI where it was „substituted" by *Hypsibius pallidus*. The mentioned study plots do not differ from the others in values of the studied environmental variables hence it is not possible to explain their difference in species diversity on this basis.

Species diversity at the other sites mostly differ from those mentioned above as well as among each other. These differences are mostly caused by species which were present in only one or two samples within series from each study plot. Only four species (*Diphascon pingue*, *Macrobiotus* cf. *harmsworthi*, *M. hufelandi* and *Minibiotus intermedius*) occurred in more than two samples in some of the study plots. So due to restricted number of samples at each study site, the occurrence of most of species seem to be random.

Tardigrada communities at the study sites

The results of Tardigrada community clustering according to pooled log-transformed abundance of species at the study sites is presented in Fig. 1.

Five clusters can be recognized on high dissimilarity level (from left to right in Fig. 1): 1 – communities on study plots CA, FU, LI, LH and HH, 2 – community on NK2, 3 – community on VI, 4 – community on LL and 5 – communities on NA and NK1.

The considerable component of the communities in the first cluster was *Macrobiotus hufelandi* (not found in the other communities). Also the species *Astatumen trinacriae* (dominancy up to 4%) and *Diphascon pingue* (dominancy app. 5–8 %) were found in all these communities. We can recognize 3 subgroups within this cluster: a – communities in CA and FU (relatively significant representation of *Diphascon prorsirostre*), b – communities in LI and LH (nearly all the found specimens of *Minibiotus intermedius*), c – community in HH (nearly all found specimens of *Macrobiotus* cf.

Table 4. Results of t-tests of Shannon-Wiener's species diversity H' for the Tardigrada communities in couples of study plots.

Study plot	CA	VI	FU	LI	HH	LL	LH	NA	NK1	NK2
Study plot/H'	0.529	0.677	0.676	0.548	1.891	1.099	1.245	0.000	0.939	1.584
CA		169	489	1663	75	3	675	931	385	44
VI	**1.600ns**		271	169	148	3	195	126	241	77
FU	2.017*	**0.011ns**		488	120	3	533	304	521	60
LI	**0.377ns**	**1.401ns**	**1.763ns**		75	3	663	753	385	44
HH	13.514***	9.504***	10.622***	13.324***		4	86	59	108	78
LL	**1.699ns**	**1.225ns**	**1.246ns**	**1.644ns**	**2.287ns**		3	3	3	4
LH	12.882***	5.908***	7.337***	12.527***	6.184***	**0.437ns**		291	433	48
NA	15.478***	7.901***	10.546***	15.926***	19.949***	3.296*	28.418***		220	38
NK1	6.043***	2.524*	3.031**	5.765***	8.549***	**0.472ns**	4.194***	16.045***		56
NK2	8.125***	5.979***	6.457***	7.981***	**1.954ns**	**1.364ns**	2.554*	12.648***	4.669***	

Notes: for abbreviations of study plots see Table 1; t-values are under the diagonal and corresponding degrees of freedom are above it; ns non significant – $P > 0.05$, * $0.01 < P < 0.05$, ** $0.001 < P < 0.01$, *** $P < 0.001$; P is probability for t-value

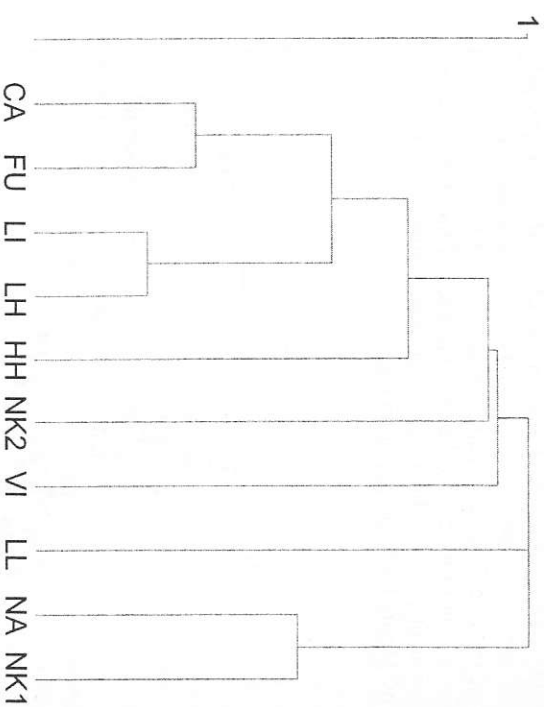

Fig. 1. Dendrogram of Tardigrada communities at the study sites (for abbreviations of study plots see Table 1; scale of dissimilarity in vertical axis).

vanescens – the richest community with 9 species). This first cluster is nearly identical with a group of study plots with statistically same species diversity (CA, VI, FU and LI). Community in VI does not belong to this cluster due to the absence of *M. hufelandi*. The differences between results of species diversities t-tests and cluster analysis are caused by the fact that species composition plays a role in cluster analysis while species may be mutually substituted in calculation and testing of species diversities.

Community in NK2 was distinguished from the others by presence of some species which were found only here (*Hypsibius* cf. *morikawai*, *Astatumen bartosi*, *Macrobiotus* cf. *seychellensis*) as well as by concentration of nearly all the specimens of *Diphascon brevipes*. Community in VI is different from the others in presence of all the specimens of *Hypsibius dujardini* and nearly all the specimens of *H. pallidus* as well as with absence of *Macrobiotus* cf. *harmsworthi* and *M. hufelandi*. Community in LL is the second poorest one with only three identified species (all present in some other communities) and also with absence of *M.* cf. *harmsworthi* as well as *M. hufelandi*. Finally, the communities in NA and NK1 are joined by meaningful proportion of *M.* cf. *harmsworthi* specimens however the community in NA contained only this species (the purest one) on the contrary to community on NK1 with 8 species (second richest one).

As we can see the studied communities embody relatively large structural differences. However, the presence of concrete species in the only sample was the cause of their mutual differentiation in some cases: *Diphascon prorsirostre* on CA and FU, *Macrobiotus* cf. *vanescens* on HH, *Hypsibius* cf. *morikawai*, *Astatumen bartosi* and

Macrobiotus cf. *seychellensis* on NK2 and *Hypsibius dujardini* and *H. pallidus* on VI. Therefore we believe that the result of cluster analysis is largely caused by random distribution as well as by apparently small number of samples at different study plots.

Environmental variables and community structure

Only two gradient variables – slope of study plot and pH of litter in H_2O – from amongst 7 gradient and 2 categorial variables were significant as explanatory (their $P < 0.05$ when tested by Monte Carlo permutation test) in the CCA when the pooled log-transformed abundances of particular species at the study plots were used as species data (Fig. 2). Eigenvalues of the two first canonical axes are $\lambda_1 = 0.614$ and $\lambda_2 = 0.340$. The first two canonical axes account for 35% of the total variance of the species data and 100% of the species-environment relation. First canonical axis is significant ($F = 2.030$, $P = 0.015$) when tested by Monte Carlo permutation test.

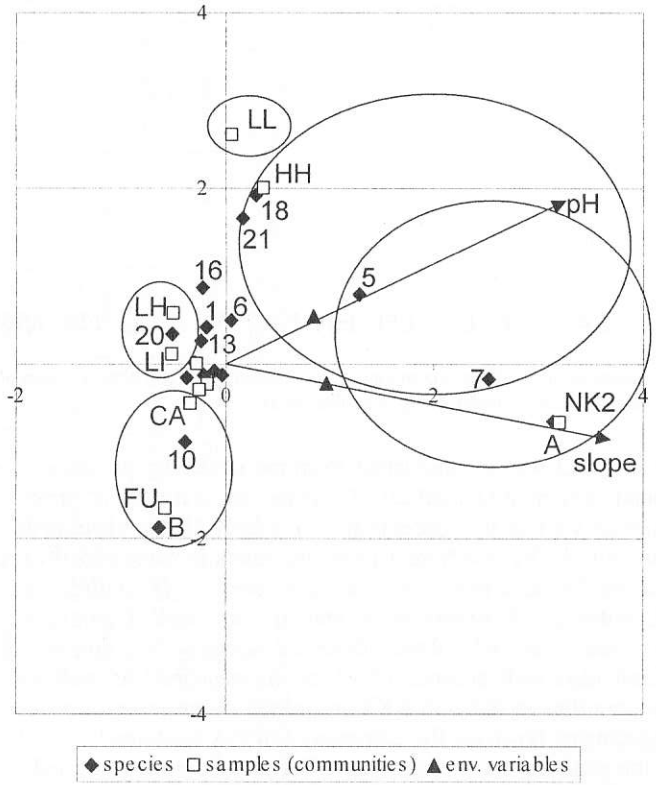

Fig. 2. Biplot of CCA of the communities at the study sites according to pooled abundance of species (for abbreviations of study plots see Table 1; A species 3, 12 and 17, B species 11 and 19; for species codes see Table 3; species found only in one or two communities are in ellipse together with appropriate community).

Variable slope was significant as it had expressively extreme value of 45° at the study plot NK2. Similarly, pH of litter in H_2O had the second maximum value 6.45 at the same study plot while the maximum value of 6.74 was recorded at the study plot LL due to calcareous dust which is produced by the nearby stone quarry. That is the reason why communities on NK2 and LL have extreme position on biplot. Among them, the community on HH with the third maximum pH value is situated. The next two communities (LL and HH) have no significant relation to slope regarding its low values being compared with that on NK2. The rest of communities are located at the other end of its scale and their location on the biplot is due to species representation and relation of their species to two gradient variables.

Similarly, the species position on biplot is not only due to their relation to two significant variables but also to their communities. For example, three species *Hypsibius* cf. *morikawai*, *Astatumen bartosi* and *Macrobiotus* cf. *seychellensis* (position A on biplot) were found only in NK2. Two other species were detected as mutual in communities in NK2 and HH: *Isohypsibius lunulatus* and *Diphascon brevipes* (see Table 3 and biplot on Fig. 2). Though it seems that three species found only in NK2 have a real relationship to localities with steep slopes it does not have to be, as each of these species was found only in one sample so it concerned accidental findings. The relation of *Isohypsibius lunulatus* and *Diphascon brevipes* to higher values of litter pH is rather different as the second species mentioned was found in three while the first one was found in four samples (on each occasion in two samples) from these two study plots. However, we are not fully convinced that dependence of these species on less acid substratum is hereby demonstrated as they lacked on study plot LL.

The result of CCA is in good agreement with the result of cluster analysis as groups of communities we can recognize in both the graphs are practically the same (Figs 1 and 2). We believe that the result of CCA was affected not only by extreme values of two significant gradient variables but also by abundance of the same species which determined the result of cluster analysis.

Ecological macrodistribution of Tardigrada *species in oak-hornbeam forests*

Only three categories of two nominal variables – study plot VI, two age intervals of forest stand 40–60 and 80–100 years – from amongst 13 variables were significant as explanatory (their $P < 0.05$ when tested by Monte Carlo permutation test) in the CCA when the log-transformed abundances of particular species in the samples were used as species data (Fig. 3). Eigenvalues of the two first canonical axes are $\lambda_1 = 0.524$ and $\lambda_2 = 0.444$. The first two canonical axes account only for 9% of the total variance of the species data and 73% of the species-environment relation. First canonical axis is significant ($F = 2.278$, $P = 0.042$ when tested by Monte Carlo permutation test).

40–60 year old forests were represented only at study sites NA and NK1 while the study plot NK2 was covered by an 80–100 year old forest. The study plot VI, together with HH, are the only plots in which the forest was 60–80 years old. So it seems that age

of a forest stand is the only one, of all the studied variables, affecting quantitative structure of tardigrade species in different moss samples.

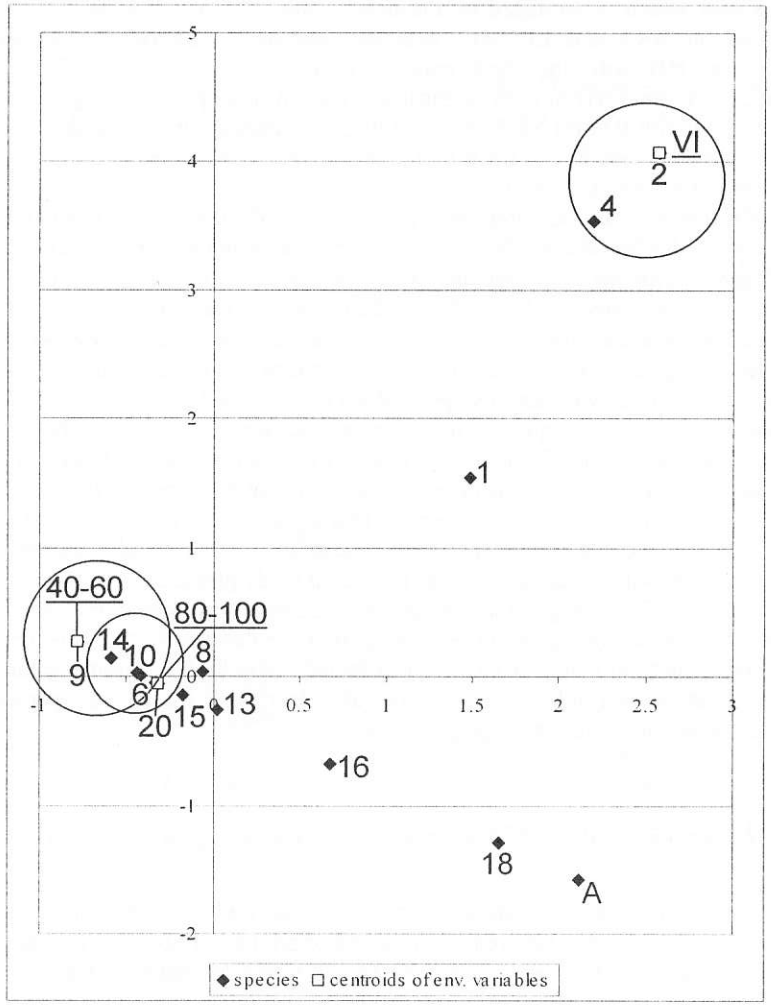

Fig. 3. Biplot of CCA of the communities at the study sites according to abundance of species in samples (VI study site Vinosady, 40–60 and 80–100 = age intervals of forest stand; A species 3, 5, 7, 12, 17 and 21; for species codes see Table 3; species found only in one or two communities are in ellipse together with appropriate community).

There were no remarkable relationships between species distribution and forest stands of a certain age interval except for a few exceptions which were seen in several species found in one random sample (*Diphacon belgicae* in 40–60 year old forest stand, *Diphascon scoticum* and *Macrobiotus* sp. 1 in 80–100 year old forest stand, *Hypsibius dujardini* and *Minibiotus* sp. 1 in 60–80 year old stand and *Hypsibius* cf. *morikawai*, *Astatumen bartosi* and *Macrobiotus* cf. *seychellensis* in 100–120 year old stand). Thus for example *Macrobiotus* cf. *harmsworthi*, *Diphascon prorsirostre*, *Isohypsibius prosostomus* and *Minibiotus intermedius* were found in 40–60 year old stands as well as in those of 80–100 years of age. Similarly, *Macrobiotus hufelandi*, *M. pallarii* and *M.* cf. *vanescens* were discovered in 60–80 years old forests as well as in those of 80–100 years of age. The other species occurred in a broader age spectrum of forest stands. From those we can mention the most constant species such as *Astatumen trinacriae* (age interval 40–100 years) and *Diphascon pingue* (all the age categories studied). It is necessary to emphasize that most of the mentioned species were found in only one sample at the sites (for example *Macrobiotus* cf. *harmsworthi* on NK2, *Diphascon prorsirostre* on FU, *Isohypsibius prosostomus* on CA, LL and NK1, *Macrobiotus pallarii* on HH and LH, *M.* cf. *vanescens* on HH and LL). On distant position A (Fig. 3), we find those species having no affinity to any from mentioned three significant categories. In addition also every from these species was found in each occasion only in one sample. Those are: *Hypsibius* cf. *morikawai*, *Isohypsibius lunulatus*, *Diphascon brevipes*, *Astatumen bartosi*, *Macrobiotus* cf. *seychellensis* and *Minibiotus* sp. 1.

We believe that two significant categories of forest stand age substitute study plots covered by those forests. Thus category 40–60 aged forest substitutes study plots NA and NK1 as well as category 80–100 aged stand substitutes study plots CA, FU, LI, LL and LH. From this visual angle, the result of this CCA is in quite agreement with the result of cluster analysis as well as with that obtained by previous CCA of tardigrades communities in study plots (Figs 1, 2).

We do not consider any of the variables to be really significant for uniqueness of findings of many tardigrade species responsible for the result of CCA. Thus we believe that water bears as passively dispersed organisms have no significant relationship to a majority (if any) of ecological variables since they dispose of sufficiently broad ecological valence to maintain in randomly colonized substrate in larger or negligible density. Variables potentially having significant influence to population density changes (species associations, humidity) were partly discussed in this study.

Translated by P. Degma

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References

Bartoš, E., 1937a: Tardigrada of Slovakia and Transcarpathia (in Czech). Bratislava, *11*, p. 250–265.

Bartoš, E., 1937b: Eine neue Tardigraden-Art aus Böhmen. Zool. Anz., *120*, p. 27–29.

Bartoš, E., 1941: Studien über die Tardigraden des Karpathengebiets. Zool. Jb., Abteilung für Systematik, Ökologie und Geographie der Tiere, *74*, p. 435–472.

Beasley, C.W., 1995: The phylum Tardigrada. McMurry University, Abilene, Texas, USA, 1014 pp.

Bertolani, R., Rebecchi, L., 1993: A revision of the *Macrobiotus hufelandi* group (Tardigrada, Macrobiotidae), with some observations on the taxonomic characters of eutardigrades. Zool. Scri., *22*, p. 127–152.

Biserov, V.I., 1990: On the revision of the *Macrobiotus* genus. The subgenus *Macrobiotus* s. str. is a new taxonomic status of the *hufelandi* group (Tardigrada, Macrobiotidae). Communication 2 (in Russian). Zool. Zhurnal, *69*, p. 38–50.

Collins, M., Bateman, L., 2001: The ecological distribution of tardigrades in Newfoundland. Zool. Anz., *240*, p. 291–297.

Dastych, H., 1980: Water bears (Tardigrada) of Tatras National Park (in Polish). Monografie Fauny Polski, *9*, Krakow, p. 1–232.

Dastych, H., 1988: The Tardigrada of Poland. Monografie Fauny Polski, *16*. Państwowe wydawnictwo naukove, Kraków, 255 pp.

Degma, P., 2003: First records of six Hypsibiidae species (Tardigrada, Eutardigrada) in Slovakia. Biologia, Bratislava, *58*, p. 1003–1005.

Degma, P., Gulánová, S., Šimurka, M., 2004: First records of three Hypsibiidae species (Tardigrada: Eutardigrada) in Slovakia. Biologia, Bratislava, *59*, Suppl. 15, p. 29–31.

Degma, P., Gulánová, S., Šimurka, M., 2005: Water bears (Tardigrada) (in Slovak). In Majzlan, O. (ed.): Fauna Devínskej Kobyly, APOP, Bratislava, p. 31–32.

Guidetti, R., Bertolani, R., 2005: Tardigrade taxonomy: an updated check list of the taxa and a list of characters for their identification. Zootaxa, *845*, p. 1–46.

Guoth, S., 1986: Tardigrada of mosses in the Little Carpathians. In Nosek, J. (ed.): The soil fauna of the Little Carpathians. Ústav experimentálnej biológie a ekológie SAV, Bratislava, p. 355–358.

Ito, M., 1999: Ecological distibution, abundance and habitat preference of terrestrial Tardigrades in various forests on the northern slope of Mt. Fuji, Central Japan. Zool. Anz., *238*, p. 225–234.

Jönsson, K.I., 2003: Population density and species composition of moss-living tardigrades in a boreo-nemoral forest. Ecography, *26*, p. 356–364.

Kathman, R.D., Cross, S.F., 1991: Ecological distribution of moss-dwelling tardigrades on Vancouver Island, British Columbia, Canada. Can. J. Zool., *69*, p. 122–129.

Kubínska, A., Mišíková, K., 1998: Bryophytes (in Slovak). In Marhold, K., Hindák, F. (eds): Checklist of non-vascular and vascular plants of Slovakia. Veda, Bratislava, p. 297–331.

Manugistics, Inc., 1993: Statgraphics Plus Version 7 for DOS. Reference Manual, 1184 pp.

Maucci, W., 1981: Analisi preliminare di alcuni dati statistici sulla ecologia dei tardigradi muscicoli. Boll. Muss. Civ. St. Nat. Verona, *7*, p. 1–47.

Mrva, M., 2005: Diversity of active gymnamoebae (Rhizopoda, Gymnamoebia) in mosses of the Malé Karpaty Mts (Slovakia). Ekológia (Bratislava), *24*, Suppl. 2, p. 51–58.

Nelson, D.R., 1975: Ecological distribution of Tardigrades on Roan Mountain, Tennessee-North Carolina. Mem. Ist. Ital. Idrobiol., *32*, Suppl., p. 225–276.

Nelson, D.R., Adkins, R.G., 2001: Distribution of tardigrades within a moss cushion: do tardigrades migrate in response to changing moisture conditions? Zool. Anz., *240*, p. 493–500.

Nichols, P.B., Romano, III., F.A., Nelson, D.R., 2001: Seasonal and altitudinal variation in the distribution and abundance of Tardigrada on Dugger Mountain, Alabama. Zool. Anz., *240*, p. 501–504.

Peters, T., Dumjahn, P., 1999: Ecological aspects of tardigrade distribution on Disko Island, West Greenland. Berichte zur Polarforschung, *330*, p. 64–75.

Pilato, G., Binda, M. G., 1999: Three new species of *Diphascon* of the *pingue* group (Eutardigrada, Hypsibiidae) from Antarctica. Polar Biology, *21*, p. 335–342.

Pilato, G., Binda, M.G., Napolitano, A., Moncada, E., 2000: The specific value of *Macrobiotus coronatus* De Barros 1942, and description of two new species of the *harmsworthi* group (Eutardigrada). Boll. Acc. Gioenia Sci. Nat., *33*, 358, p. 103–120.

Podani, J., 1993: Syn-Tax. Version 5.0. Computer Programs for Multivariate Data Analysis in Ecology and Systematics. User's guide. Scientia Publishing, Budapest, 104 pp.

Poole, R.W., 1974: An Introduction to Quantitative Ecology. McGraw-Hill, 532 pp.

Ramazzotti, G., Maucci, W., 1983: Il Philum Tardigrada. (III. edizione riveduta e aggiornata). Memoire dell' Istituto Italiano di Idrobiologia, *41*, 1016 pp.

Romano, III, F.A., Barreras-Borrero, B., Nelson, D.R., 2001: Ecological distribution and community analysis of Tardigrada from Choccolocco Creek, Alabama. Zool. Anz., *240*, p. 535–541.

Spellerberg, I.F., Fedor, P.J., 2003: A tribute to Claude Shannon (1916-2001) and a plea for more rigorous use of species richness, species diversity and the "Shannon-Wiener" Index. Global Ecology and Biogeography, *12*, 3, p. 177–179.

Steiner, W. A., 1994: The influence of air pollution on moss-dwelling animals: 4. Seasonal and long-term fluctuations of rotifer, nematode and tardigrade populations. Rev. Suisse Zool., *101*, p. 1017–1031.

Ter Braak, C.J.F., Šmilauer, P., 1998: CANOCO References Manual and User's Guide to Canoco for Windows: Software for Canonical Community Ordination (version 4). Microcomputer Power (Ithaca, NY, USA), 352 pp.

Wright, J. C., 1991: The significance of four xeric parameters in the ecology of terrestrial Tardigrada. J. Zool., *224*, p. 59–77.

Zlinská, J., Šomšák, L., Holecová, M., 2005: Ecological characteristics of studied forest communities of an oak-hornbeam tier in SW Slovakia. Ekológia (Bratislava), *24*, Suppl. 2, p. 3–19.

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Degma P., Šimurka M., Gulánová S.: **Štruktúra spoločenstva a ekologická makrodistribúcia pomaliek (Tardigrada) žijúcich v machoch stredoeurópskych dubovo-hrabových lesov (JZ Slovensko).**

Študovali sme štruktúru spoločenstiev pomaliek (Tardigrada) v machoch stredoeurópskych dubových lesov na 10 študijných plochách situovaných v Malých Karpatoch a Trnavskej pahorkatine (JZ Slovensko).

Zo 79 kvantitatívnych vzoriek odobratých v rokoch 2000 a 2002 sme získali 3 050 jedincov pomaliek 21 druhov a dvoch čeľadí.

Kruskal-Wallisove testy a regresné analýzy ukázali, že žiadna z 12 študovaných environmentálnych premenných nemá štatisticky významný vplyv na počet jedincov alebo druhov Tardigrada vo vzorkách. Chí-kvadrátový test dobrej zhody naznačil, že počet druhov Tardigrada vo vzorkách machu *Hypnum cupressiforme* bol v rámci skúmanej oblasti náhodný.

Výsledkom t-testu druhovej diverzity boli významné rozdiely medzi študijnými plochami. Skupina spoločenstiev s najnižšou diverzitou sa vzájomne nelíši. Väčšinu odlišností v druhovej diverzite spôsobili náhodne nájdené druhy.

Výsledky zhlukovej analýzy ako aj CCA poukazujú na to, že šírenie pomaliek a ich osídľovanie jednotlivých substrátov je náhodný proces.

Hoci sú výsledky ovplyvnené obmedzeným počtom vzoriek, domnievame sa, že Tardigrada ako pasívne sa šíriace organizmy majú nevýznamnú väzbu na ekologické premenné súvisiace s ich distribúciou medzi substrátmi.